appropriate for insertion of the negative selection marker in the Y chromosome of mammalian male F1 ES cells, thereby producing a mixture of mammalian male F1 ES cells comprising male F1 ES cells in which the negative selection marker in inserted in the Y chromosome and other male F1 ES cells, some of which do not contain a Y chromosome; subjecting the resulting mixture to conditions that result in the death of male F1 ES cells in which the Y chromosome has the negative selection marker inserted therein and do not result in the death of male F1 ES cells that lack a Y chromosome and are XO F1 ES cells, thereby producing mammalian XO F1 ES cells.

REMARKS

Information Disclosure Statement

A Second Supplemental Information Disclosure Statement (IDS) was filed on August 27, 2002. Entry and consideration of the IDS are respectfully requested.

Restriction Requirement

Applicants thank the Examiner for withdrawal of the restriction requirement.

Rejection of Claims 1, 5, 8, 11, 14, 18, 21 and 41 Under 35 U.S.C. 112, First Paragraph

Claims 1, 5, 8, 11, 14, 18, 21 and 41 have been rejected under 35 U.S.C. § 112, first paragraph, because, in the Examiner's assessment, the specification "does not reasonably provide enablement for a method of producing any non-human mammal" by injecting non-inbred ES cells into tetraploid embryos. Applicants respectfully disagree with this assessment.

The standard for enablement under 35 U.S.C. § 112, first paragraph, is whether the claimed invention can be practiced without undue experimentation given the guidance presented in the specification and what was known to the skilled artisan at the time the subject application was filed. The specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art would be able to practice it without undue experimentation. In re Borkowski, 164 U.S.P.Q. 642, 645 (C.C.P.A. 1970). See also M.P.E.P. § 2164.02.

A specification which contains a teaching of how to make and use the full scope of the claimed invention must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. In re Marzocchi, 169 U.S.P.Q. 367, 370 (C.C.P.A. 1971).

The specification teaches methods of producing non-human mammals, which can be mutant non-human mammals or non-mutant non-human mammals. The specification teaches methods of producing non-human embryos, which can be mutant non-human embryos or non-mutant non-human embryos. The specification teaches that these non-human mammals and embryos can be produced by tetraploid blastocyst complementation using non-inbred pluripotent cells, such as non-inbred ES cells (see, e.g., page 2, lines 12-15). In particular, the specification teaches that non-human mammals can be produced by introducing non-inbred pluripotent cells, such as non-inbred ES cells, into tetraploid blastocysts of the same mammalian species to produce an embryo and transferring the resulting embryo into an appropriate foster mother, such as a pseudopregnant female of the same mammalian species (see, e.g., page 6, lines 2-6). The resulting female is maintained under conditions that result in development of live offspring, thereby producing a non-human mammal derived from a single zygote, that which originally gave rise to the non-inbred pluripotent cells (see, e.g., page 6, lines 6-10). The specification teaches that mutant non-human mammals can be produced by introducing non-inbred pluripotent cells, such as non-inbred ES cells, comprising at least one mutation or alteration into tetraploid blastocysts of the same mammalian species to produce an embryo and transferring the resulting embryo into an appropriate foster mother, such as a pseudopregnant female of the same mammalian species (see, e.g., page 6, lines 12-17). The resulting female is maintained under conditions that result in development of live offspring, thereby producing a mutant non-human mammal derived from a single zygote, that which originally gave rise to the non-inbred pluripotent cells (see, e.g., page 6, lines 17-22). The specification teaches that non-human embryos can be produced by introducing non-inbred pluripotent cells, such as non-inbred ES cells, into non-human tetraploid blastocysts and maintaining the resulting tetraploid blastocysts under conditions that result in formation of embryos (see, e.g., page 9, line 28 to page 9, line 2).

Methods for producing tetraploid blastocysts and for introducing non-inbred pluripotent cells into tetraploid blastocysts were also readily available in the art at the time the subject application was filed. Examples of methods for introducing non-inbred pluripotent cells into tetraploid blastocysts are also provided in the specification (see, e.g., page 7, line 29 to page 8, line 1).

Methods for producing mutant non-inbred pluripotent cells that are used to produce mutant non-human mammals were readily available in the art at the time the subject application was filed. Examples of methods for producing mutant non-inbred pluripotent cells are also provided in the specification (see, e.g., page 9, lines 10-19).

In the Examples, Applicants provide evidence that non-mutant non-human mammals and mutant non-humans mammals are produced by tetraploid blastocyst complementation using non-inbred pluripotent cells as described in the specification. In particular, applying the teachings of the specification, both non-mutant and mutant mice were produced by tetraploid blastocyst complementation using non-inbred pluripotent cells (see, e.g., page 12, line 6 to page 16, line 10; page 19, Table II). Applicants have thus demonstrated that both non-mutant and mutant non-human mammals are produced by tetraploid blastocyst complementation using non-inbred pluripotent cells when following the written disclosure.

Thus, armed with the teachings in the specification and what was known to the skilled artisan at the time the subject application was filed, it would have been a routine matter for one skilled in the art to produce non-mutant non-human mammals, mutant non-human mammals, non-mutant non-human embryos and mutant non-human embryos by tetraploid blastocyst complementation using non-inbred pluripotent cells. Accordingly, Applicants submit that the guidance provided in the specification, coupled with what was known to the skilled artisan at the time the subject application was filed, is sufficient to enable the skilled artisan to make and use the full scope of the subject matter of Claims 1, 5, 8, 11, 14, 18, 21 and 41.

The Examiner appears to doubt that the teachings in the specification are sufficient to enable one skilled in the art to practice the full scope of the claimed invention without undue experimentation because ES cell technology was known only in the mouse and "the skilled artisan did not accept that it was possible to have prepared ES cells in other species". Paper No. 8, at page 5, lines 15-18. The Examiner points to Bradley *et al.* (*Bio/Technology*, 10:534-

539 (1992)) and Campbell *et al.* (*Theriogenology*, 47:63-72 (1997)) as providing evidence in support of this position. Applicants respectfully traverse.

The Bradley *et al.* reference, which was published in 1992, approximately 8 years before the September 20, 2000 effective filing date of the subject application, reviews several techniques used to isolate targeted clones of ES cells in 1992 and several strategies used to make specific genome modifications in 1992. While Bradley *et al.* indicate that in 1992, it had yet to demonstrate that ES cells isolated from farm animal species (pigs, sheep) can proliferate and differentiate in an embryo *in vivo* and contribute to somatic tissues or germ cells, the authors do not conclude that the ES cells isolated from farm animal species are not ES cells or that ES cells cannot be isolated from mammalian species other than mice. In fact, Bradley *et al.* indicate that "ES cells offer the same potential advantages for genetic engineering of large animals that have been realized in mice" (Bradley *et al.*, page 538, lines 2-7). This suggests that one skilled in the art would reasonably expect to be able to isolate ES cells from mammalian species other than mice. Accordingly, Bradley *et al.* do not provide a sufficient basis to question the enablement provided in the subject specification for the full scope of Claims 1, 5, 8, 11, 14, 18, 21 and 41.

The Campbell *et al.* reference, which was published in 1997, describes methods of embryo manipulation, the properties of cells required for the production of chimeric and nuclear transfer animals and potential advantages/disadvantages of these methods for the stable introduction of new genetic material in farm animal species. As the Examiner notes, Campbell *et al.* acknowledge reports of ES-like cell lines in a number of mammalian species, including pigs, sheep, cattle and primate. While Campbell *et al.* indicate that "as yet there are no reports of any cell lines which contribute to germ line in any species other than the mouse", the reference does not conclude that ES cells cannot be isolated from mammalian species other than mice or provide evidence that would lead one skilled in the art to the conclusion that Applicants' claimed method of producing non-mutant non-human mammals, mutant non-human mammals, non-mutant non-human embryos and mutant non-human embryos by tetraploid blastocyst complementation using non-inbred pluripotent cells cannot be practiced on any species other than the mouse. Accordingly, Campbell *et al.* do not provide a sufficient basis to question the enablement provided in the subject specification for the full scope of Claims 1, 5, 8, 11, 14, 18, 21 and 41.

Moreover, Thomson *et al.* (*Proc. Natl. Acad. Sci. USA*, 92:7844-7848 (1995); attached hereto as Exhibit 1), Cibelli *et al.* (*Nature Biotechnology*, 16:642-646 (1998); attached hereto as Exhibit 2) and Iwasaki *et al.* (*Biol. Reprod.*, 62:470-475 (Feb. 2000); attached hereto as Exhibit 3) provide evidence that Applicants' specification enables one skilled in the art to make and use full scope of the claimed invention of Claims 1, 5, 8, 11, 14, 18, 21 and 41, particularly with mammalian species other than the mouse. In particular, Thomson *et al.* report the isolation of an ES cell line from a rhesus monkey blastocyst that remains undifferentiated and continues to proliferate for greater than 1 year in culture, maintains a normal XY karyotype, and maintains the potential to differentiate into trophoblast and to derivatives of embryonic endoderm, mesoderm and ectoderm. Cibelli *et al.* report the production of pluripotent ES-like cells from bovine embryos that retain the ability to differentiate into embryonic endoderm, mesoderm and ectoderm in offspring. Iwasaki *et al.* report the production of chimeric calves from bovine ES-like cells aggregated with tetraploid embryos.

Reconsideration and withdrawal of this rejection of Claims 1, 5, 8, 11, 14, 18, 21 and 41 under 35 U.S.C. § 112, first paragraph, are respectfully requested.

Rejection of Claim 40 Under 35 U.S.C. 112, First Paragraph

Claim 40 has been rejected under 35 U.S.C. § 112, first paragraph, because, in the Examiner's assessment, the claim contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and use the invention. The Examiner alleges that it would require undue experimentation for the skilled artisan to practice the claimed invention because the specification does not provide any guidance as to (1) "which drugs are tested using the mouse model system; (2) "which conditions can be treated using the said drugs"; (3) "in which mammal the said condition occurs"; (4) "which of the candidate drugs is useful in treating or preventing a specific condition in a specific mammal"; (5) "how one of skill in the art can use the method of the instant invention to produce any mammal such that any drug can be tested for its effectiveness and ability to treat or prevent a condition in the said mammal"; (6) "the phenotypes of the claimed mammals with the claimed condition or conditions such that one skilled in the art would know how to evaluate the effectiveness of the said drug in treating or preventing the said condition in any mammal". Paper No. 8, at page 7, lines 5-16.

Applicants respectfully disagree with the Examiner's assessment that the subject matter of Claim 40 is not enabled.

Claim 40 is limited to use of a mutant mouse that is a model of a condition that occurs in a different mammalian species to identify candidate drugs that have a therapeutic or preventive effect on the condition.

The specification teaches that a candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal can be identified by using a mutant mouse that is a model of the condition to screen drugs for the ability to treat or prevent the condition. More specifically, the specification teaches that a candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal can be identified by producing a mutant mouse model of the condition in accordance with the methods of the invention, administering to the mutant mouse model a drug to be assessed, and assessing the ability of the drug to treat or prevent the condition in the mutant mouse model (see, e.g., page 11, lines 24-29). If the drug reduces the extent to which the condition is present or progresses or causes the condition to reverse in the mutant mouse model of the condition, the drug is identified as a candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal (see, e.g., page 11, line 29 to page 12, line 3).

The specification teaches that a mutant mouse that is a model of a condition for which a preventive or therapeutic drug is sought can be produced by introducing mouse non-inbred pluripotent cells, such as mouse non-inbred ES cells, comprising at least one mutation or alteration in genomic DNA into mouse tetraploid blastocysts to produce a mouse blastocysts containing mouse non-inbred pluripotent cells, maintaining the mouse blastocysts containing non-inbred pluripotent cells under conditions for production of embryos, and transferring the resulting embryo into an appropriate foster mother, such as a pseudopregnant female mouse (see, e.g., page 6, lines 23-29; page 3, lines 25-28; and page 11, lines 23-24). The resulting female is maintained under conditions that result in development of live offspring, thereby producing a mutant mouse (see, e.g., page 7, lines 1-2).

The specification teaches that the mutant mouse model serves as a model of a condition that occurs in a different mammalian species (see, e.g., page 3, lines 25-28). Such a condition includes neurological, muscular or respiratory conditions, cancer, viral infection, arthritis (see,

e.g., page 3, lines 25-27), and conditions caused by or associated with a genetic alteration (see, e.g., page 4, lines 4-5). Such conditions, along with defining characteristics (symptoms, signs, pathology, phenotypes, etc.) were well known and described in the art at the time the subject application was filed. See, e.g., Beers *et al.* (Eds.), *The Merck Manual of Diagnosis and Therapy*, 17th edition, Merck Research Laboratories (1999) (for human conditions). Mouse models of conditions that occur in different mammalian species were known and described in the art at the time the subject application was filed. Mouse models can also be designed using information and methods that were readily available in the art at the time the subject application was filed. See, e.g., Popko (Ed.), *Mouse Models in the Study of Genetic Neurological Disorders (Advances in Neurochemistry, V. 9)*, 1st edition, Kluwer Academic Publishers (1999); Tarui *et al.* (Eds.), *Insulitis and Type I Diabetes: Lessons from the Nod Mouse*, Academic Press (1997); Mohr *et al.* (Eds.), *Cancer Pathology of Tumours in Laboratory Animals: Tumours of the Mouse (Iarc Scientific Publications, No. 111)*, 2nd edition, Oxford University Press (1994); and Sundberg (Ed.), *Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools*, CRC Press (1994). The specification is not required to disclose what is well known in the relevant art at the time the subject application was filed. See, e.g., Lindemann Maschinenfabrik G.m.b.H. v. American Hoist & Derrick Co., 221 U.S.P.Q. 481, 489 (Fed. Cir. 1984); Hybritech, Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and In re Wands, 8 U.S.P.Q.2d 1400, 1402 (Fed. Cir. 1988).

The Examiner appears to doubt that the teachings in the specification are sufficient to enable one skilled in the art to practice the subject matter claimed in Claim 40 because "the specification does not provide any guidance as to which drugs are tested using the mouse model system". Paper No. 8, at page 7, lines 5-6. The Examiner has not provided any evidence or reasoning in support of this position.

Claim 40 relates to a method of *identifying* a candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal by using a mutant mouse that is a model of the condition to screen drugs for the ability to treat or prevent the condition, wherein the mutant mouse is produced by tetraploid blastocyst complementation using mouse non-inbred pluripotent cells as described in the specification.

The method of Claim 40 is not predicated on the identity of the candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal. The method is predicated on the ability to screen and identify candidate drugs having a therapeutic or preventive effect on a condition that occurs in a mammal in accordance with the claimed method. That is, the method of Claim 40 is predicated on the ability to screen for the ability (+) or inability (-) of a particular drug to treat or prevent the condition in a mutant mouse model of the condition. If the drug reduces the extent to which the condition is present or progresses or causes the condition to reverse in the mutant mouse model of the condition, the drug is identified as a candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal. There is no basis to question that the skilled artisan, armed with Applicants' teachings, could practice, without undue experimentation, the claimed method of identifying a candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal.

Accordingly, armed with Applicants' teachings in the specification, it would be a routine matter to identify a candidate drug having a therapeutic or preventive effect on a condition that occurs in a mammal by using a mutant mouse that is a model of the condition to screen drugs for the ability to treat or prevent the condition, wherein the mutant mouse is produced by tetraploid blastocyst complementation using mouse non-inbred pluripotent cells as described in the specification.

Reconsideration and withdrawal of this rejection of Claim 40 under 35 U.S.C. § 112, first paragraph, are respectfully requested.

Rejection of Claim 44 Under 35 U.S.C. 112, First Paragraph

Claim 44 has been rejected under 35 U.S.C. § 112, first paragraph, because, in the Examiner's assessment, the specification "does not reasonably provide enablement for a method of producing XO F1 ES cells in any mammal and or any organism". Applicants respectfully disagree with this assessment.

As amended, Claim 44 relates to a method of producing mammalian XO F1 ES cells.

The specification teaches a method of producing mammalian XO F1 ES cells by introducing a negative selection marker into mammalian male F1 ES cells under conditions appropriate for insertion of the negative selection marker in the Y chromosome of mammalian

male F1 ES cells, thereby producing a mixture of male F1 ES cells with the negative selection marker inserted in the Y chromosome and male F1 ES cells that lack a Y chromosome (see, e.g., page 5, lines 14-18; and page 10, lines 18-27). The resulting mixture of mammalian male F1 ES cells is subjected to conditions that result in death of male F1 ES cells with the negative selection marker inserted in the Y chromosome and do not result in death of male F1 ES cells that lack a Y chromosome, thereby producing mammalian XO F1 ES cells (see, e.g., page 5, lines 18-19; and page 10, line 28 to page 11, line 1). Such conditions were known and readily available in the art at the time the subject application was filed.

The specification teaches that the negative selection marker can be inserted onto the Y chromosome by homologous recombination using methods known and readily available in the art at the time the subject application was filed (see, e.g., page 10, lines 20-22). For example, a vector that contains sequences homologous to a Y-linked gene and expresses the negative selection marker can be produced and introduced into F1 ES cells (see, e.g., page 10, lines 22-24). Negative selection markers were known and readily available in the art at the time the subject application was filed.

Thus, armed with the teachings in the specification and what was known to the skilled artisan at the time the subject application was filed, it would have been a routine matter for one skilled in the art to produce mammalian XO F1 ES cells by introducing a negative selection marker into mammalian male F1 ES cells under conditions appropriate for insertion of the negative selection marker in the Y chromosome of mammalian male F1 ES cells, thereby producing a mixture of male F1 ES cells with the negative selection marker inserted in the Y chromosome and male F1 ES cells that lack a Y chromosome, and subjecting the resulting mixture of mammalian male F1 ES cells to conditions that result in death of male F1 ES cells with the negative selection marker inserted in the Y chromosome and do not result in death of male F1 ES cells that lack a Y chromosome, thereby producing mammalian XO F1 ES cells.

The Examiner appears to doubt that the teachings in the specification are sufficient to enable one skilled in the art to practice the full scope of the subject matter of Claim 44 without undue experimentation because "ES cell technology is available only in mice" and "ES cells and their use in generating adult organisms is well known only in the case of mice". Paper No. 8,

lines 3-10. It is believed that Bradley *et al.* and Campbell *et al.* are again relied upon by the Examiner as providing evidence in support of this position. Applicants respectfully traverse.

As discussed above, while Bradley *et al.* indicate that in 1992 (approximately 8 years before the September 20, 2000 effective filing date of the subject application), it had yet to demonstrate that ES cells isolated from farm animal species (pigs, sheep) can proliferate and differentiate in an embryo *in vivo* and contribute to somatic tissues or germ cells, the authors do not conclude that the ES cells isolated from farm animal species are not ES cells or that ES cells cannot be isolated from mammalian species other than mice. In fact, Bradley *et al.* indicate that "ES cells offer the same potential advantages for genetic engineering of large animals that have been realized in mice" (Bradley *et al.*, page 538, lines 2-7). This suggests that one skilled in the art would reasonably expect to be able to isolate ES cells from mammalian species other than mice. Accordingly, Bradley *et al.* do not provide a sufficient basis to question the enablement provided in the subject specification for the full scope of Claim 44.

Campbell *et al.* report ES-like cell lines in a number of mammalian species, including pigs, sheep, cattle and primate. While Campbell *et al.* indicate that "as yet there are no reports of any cell lines which contribute to germ line in any species other than the mouse", the reference does not conclude that ES cells cannot be isolated from mammalian species other than mice or provide evidence that would lead one skilled in the art to the conclusion that Applicants' claimed method of producing mammalian XO F1 ES cells cannot be applied to mammalian male F1 ES cells other than mouse male F1 ES cells. Accordingly, Campbell *et al.* do not provide a sufficient basis to question the enablement provided in the subject specification for the full scope of Claim 44.

Moreover, as discussed above, Thomson *et al.* (attached hereto as Exhibit 1), Cibelli *et al.* (attached hereto as Exhibit 2) and Iwasaki *et al.* (attached hereto as Exhibit 3) provide evidence that Applicants' specification enables one skilled in the art to practice the full scope of the subject matter of Claim 44, particularly with mammalian male F1 ES cell other than mouse male F1 ES. In particular, Thomson *et al.* report the isolation of an ES cell line from a rhesus monkey blastocyst that remains undifferentiated and continues to proliferate for greater than 1 year in culture, maintains a normal XY karyotype, and maintains the potential to differentiate into trophoblast and to derivatives of embryonic endoderm, mesoderm and ectoderm. Cibelli *et al.*

report the production of pluripotent ES-like cells from bovine embryos that retain the ability to differentiate into embryonic endoderm, mesoderm and ectoderm in offspring. Iwasaki *et al.* report the production of chimeric calves from bovine ES-like cells aggregated with tetraploid embryos.

Accordingly, Applicants submit that the guidance provided in the specification, coupled with what was known to the skilled artisan at the time the subject application was filed, is sufficient to enable the skilled artisan to make and use the full scope of the subject matter of Claim 44. Reconsideration and withdrawal of this rejection of Claim 44 under 35 U.S.C. § 112, first paragraph, are respectfully requested.

Rejection of Claims 7, 40 and 41 Under 35 U.S.C. § 112, Second Paragraph

Claims 7, 40 and 41 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Certain claims have been amended in response to the rejection. As amended, the claims even more particularly point out and distinctly claim the subject matter which Applicants regard as the invention, thereby obviating this rejection under 35 U.S.C. § 112, second paragraph.

As amended, the claims indicated include the following changes, made in response to the specific rejections made by the Examiner:

a) Claim 7 has been rejected as indefinite because, in the Examiner's assessment, the limitation "mutant mouse non-inbred ES cells" lacks sufficient antecedent basis.

Claims 6 and 7 have been amended to provide antecedent basis for the mutant mouse non-inbred ES cells recited in Claim 7, thereby obviating this aspect of the rejection under 35 U.S.C. § 112, second paragraph.

b) Claim 40 has been rejected as indefinite in the recitation of the phrase "to treat a condition in a mammal in which the condition occurs, comprising producing, using the method of claim 14, a mutant mouse that is a model of the condition..." because, in the Examiner's assessment, "[a] broad range or limitation together with a narrow range or limitation that falls

within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired." In particular, the Examiner alleges that Claim 40 is indefinite because the claim includes the broad recitation of "a mammal" and the recitation of "a mouse", which is the narrower statement of the range/limitation. Applicants respectfully disagree with the Examiner's assessment that recitation of "a mammal" and "a mouse" renders the metes and bounds of the instant claim unclear.

The test for definiteness is whether one skilled in the art would understand the metes and bounds of the claim when read in light of the specification. Orthokinetics, Inc. v. Safety Travel Chairs, Inc., 1 U.S.P.Q.2d 1081, 1088 (Fed. Cir. 1986). If the claims read in light of the specification reasonably appraise those skilled in the art of the scope of the invention, § 112 demands no more. Hybritech, Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81 (Fed. Cir. 1987), *cert. denied*, 480 U.S. 947 (1987).

The specification teaches that mutant non-human mammals, such as mutant mice, can be produced, using the method of the invention, to mimic or serve as a model for a condition that occurs in another species (see, e.g., page 3, lines 25-28; and page 11, lines 23-24). The specification teaches that the resulting mutant non-human mammals, such as mutant mice, are used to identify drugs that have a therapeutic or preventive effect on the condition (see, e.g., page 3, line 28 to page 4, line 1). As such, the skilled artisan would understand that mutant mice can be produced using the method of the invention to mimic or serve as a model for a condition that occurs in a *different* mammalian species and that the resulting mutant mice can be used in a method to identify drugs that have a therapeutic or preventive effect on the condition that occurs in another mammalian species, thereby identifying candidate drugs to be administered to treat the condition in a mammal.

Accordingly, the skilled artisan would understand what is claimed with respect to using "a mutant mouse that is a model of [a] condition" in a method to identify a candidate drug to be administered "to treat [the] condition in a mammal", when read in light of the specification. One skilled in the art would be reasonably apprised of the metes and bounds of Claim 40, when read in light of the specification.

Claim 40 has also been rejected as indefinite in the recitation of the phrase "the condition" and in the recitation of the phrase "a drug to be administered".

As suggested by the Examiner, Claim 40 has been amended to recite "said condition" in place of "the condition" and to recite "a candidate drug to be administered" in place of "a drug to be administered", thereby obviating this aspect of the rejection under 35 U.S.C. § 112, second paragraph.

c) Claim 41 has been rejected as indefinite in the recitation of the phrase "a mutant non-human mammal, mammal" because, in the Examiner's assessment, it is not clear to what Applicants are referring.

Claim 41 has been amended to delete the inadvertent second occurrence of the word "mammal", thereby obviating this aspect of the rejection under 35 U.S.C. § 112, second paragraph.

Rejection of Claims 1-39 and 41 Under 35 U.S.C. § 103(a)

Claims 1-39 and 41 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Wang *et al.* (*Mechanisms of Development*, 62:137-145 (1997)) in view of Rideout *et al.* (*Nature Genetics*, 24:109-110 (2000)).

Teachings of the Cited References

Wang *et al.*

Wang *et al.* is cited by the Examiner as teaching "generation of ES cell-derived mutant mice using tetraploid blastocyst injection". Paper No. 8, at page 11, lines 16-17.

As noted by the Examiner, Wang *et al.* do not teach or suggest the use of non-inbred pluripotent cells, including non-inbred ES cells, in the generation of mutant and non-mutant mice. Paper No. 8, at page 11, lines 18-19.

Rideout *et al.*

Rideout *et al.* is cited by the Examiner as teaching "the use of F1 ES cells . . . in the generation of mice and teach that genetic background has an effect on survival". Paper No. 8, at page 11, lines 20-22. Importantly, Rideout *et al.* teach the generation of mice from wild-type and targeted ES cell by nuclear cloning.

Rideout *et al.* do not teach or suggest the generation of mice using tetraploid blastocyst injection. As such, Rideout *et al.* do not cure the deficiencies of the Wang *et al.* reference.

Combination of References

In support of the rejection, the Examiner alleges that:

> [I]t would have been obvious to one of ordinary skill in the art at the time the invention was made, to use F1 ES cells to inject tetraploid blas[t]ocysts and generate mice, with a reasonable expectation of success. The motivation to do so was provided by Rideout et al. (2000) who teach that genetic background is probably an important factor in cloning efficiency and establish that F1 ES cells are efficient donor cells for generating cloned mice . . . and that manipulating the F1 ES cells in vitro before cloning may allow difficult questions such as the collective role of imprinted genes in mammalian development to be addressed.

Paper No. 8, at page 11, line 20 to page 12, line 7.

Applicants respectfully submit that this rejection is improper because the Examiner has not identified a suggestion in the prior art of the desirability of the proposed combination of references. The basis in the Rideout *et al.* reference relied upon by the Examiner for the desirability of the proposed combination of references would not have led one of ordinary skill in the art to combine the teachings of Rideout *et al.* with the teachings of Wang *et al.* to obtain the claimed invention. Wang *et al.* teach the generation of ES-derived mice by tetraploid blastocyst injection, but do not teach or suggest using non-inbred pluripotent cells, including non-inbred ES cells. Rideout *et al.* teach the generation of ES-derived mice by nuclear cloning using F1 (non-inbred) ES cells, but do not teach or suggest the generation of mice using tetraploid blastocyst injection. Combining the elements of separate references which do not themselves suggest the combination necessary to obtain a claimed invention is generally improper. ACS Hospital

Systems, Inc. v. Montefiore Hospital, 221 U.S.P.Q. 929, 933 (Fed. Cir. 1984). The only document of record which suggests the desirability of the proposed combination is Applicants' specification. However, the use of the present specification as an instruction manual or template to piece together the teachings of the prior art is impermissible hindsight.

A *prima facie* case of obviousness is established only if the teachings of the cited art would have suggested the claimed invention to one of ordinary skill in the art with a reasonable expectation of successfully achieving the claimed results. In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Both the suggestion and the reasonable expectation of success must be found in the prior art, not Applicants' disclosure. Id.

The Court of Appeals for the Federal Circuit has stated that "[t]he proper approach to the obviousness issue must start with the claimed invention *as a whole*." See, e.g., Kimberley-Clark Corp. v. Johnson & Johnson Co., 223 U.S.P.Q. 603, 609 (Fed. Cir. 1984). See also Lindemann Maschinenfabrik G.m.b.H. v. American Hoist & Derrick Co., 221 U.S.P.Q. 481, 488 (Fed. Cir. 1984). It is not proper to pick and choose among individual elements of assorted prior art references to recreate the claimed invention. Smithkline Diagnostics Inc. v. Helena Laboratories Corp., 8 U.S.P.Q.2d 1468, 1475 (Fed. Cir. 1988); Akzo N.V. v. International Trade Comm., 11 U.S.P.Q.2d 1241, 1246 (Fed. Cir. 1986).

Claims 1-39 and 41 relate to methods of producing non-human mammals and embryos (mutant non-human mammals and embryos and non-mutant non-human mammals and embryos), such as mice and mouse embryos, by tetraploid blastocyst complementation using non-inbred pluripotent cells, such as non-inbred ES cells.

Neither the Wang *et al.* reference nor the Rideout *et al.* reference, alone or in combination, would have suggested the claimed invention to one of ordinary skill in the art at the time the invention was made with a reasonable expectation of success. More specifically, one of ordinary skill in the art would not have been able to predict with a reasonable expectation of success, given the combination of cited references, whether non-human mammals and embryos (mutant and non-mutant), such as mice and mouse embryos, would be produced by tetraploid blastocyst complementation using non-inbred pluripotent cells, such as non-inbred ES cells.

As discussed above, Wang *et al.* teach the generation of ES-derived mice by tetraploid blastocyst injection, but do not teach or suggest using non-inbred pluripotent cells, including

non-inbred ES cells. Rideout *et al.* teach the generation of ES-derived mice by nuclear cloning using F1 (non-inbred) ES cells, but do not teach or suggest the generation of mice using tetraploid blastocyst injection. Accordingly, the cited references, either alone or in combination, would not have suggested the claimed invention to one of ordinary skill in the art, at the time the invention was made, with a reasonable expectation of success. At best, the cited references merely indicate that specific isolated elements and/or features recited in the claims are known. This is insufficient to render the claimed invention *prima facie* obvious.

Further, even assuming, *arguendo*, that a *prima facie* case of obviousness exists, which it does not, the *prima facie* case of obviousness would be overcome by a showing of unexpected results. It is well settled that a patent applicant can rebut a *prima facie* case of obviousness by a showing of "unexpected results", e.g., by showing that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the art would have found surprising or unexpected. See, e.g., In re Soni, 34 U.S.P.Q.2d 1684, 1687 (Fed. Cir. 1995).

Applicants obtained the unexpected and surprising result that 60 pups were produced after injection of 344 tetraploid blastocysts with 6 different ***non-inbred*** ES cell lines (i.e., 6 different F1 ES cell lines) and that 51 (85%) survived to adulthood (see, e.g., page 15, lines 18-20; and page 19, Table 2). In contrast, 20 pups were produced after injection of 312 tetraploid blastocysts with 4 different ***inbred*** ES cell lines and only 1 survived to adulthood (see, e.g., page 15, lines 13-18; and page 18, Table 1). The magnitude of these results using non-inbred ES cells (i.e., the number of pups that were produced (60) and the number of pups that survived to adulthood (51)), relative to the number of pups that were produced using inbred ES cells (20) and that survived to adulthood (1), could not have been predicted from the cited references.

Moreover, Applicants demonstrated the unexpected and surprising result that live, adult mice, entirely derived from ES cells can be generated from F1 ES cells even after long-term passage of the cells in culture or after consecutive rounds of drug selection (see, e.g., page 16, lines 7-10). In particular, Applicants found that no impairment of the resulting ES cell-tetraploid pups was noted after either 15 or 25 passages (see, e.g., page 15, lines 27-28) or after 2 consecutive rounds of drug selection (see, e.g, page 16, lines 1-7). The magnitude of these results could not have been predicted from the cited references, particularly since, as reported in

the specification, it has previously been shown that continued passage of ES cells is detrimental to their developmental potency (see, e.g., page 15, lines 22-24).

Accordingly, even assuming, *arguendo*, that a *prima facie* case of obviousness exists, which it does not, it has been overcome by Applicants' unexpected results. Reconsideration and withdrawal of the rejection of Claims 1-39 and 41 under 35 U.S.C. § 103(a) are respectfully requested.

Rejection of Claims 42-48 Under 35 U.S.C. § 103(a)

Claims 42-48 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Wang *et al.* and Uchida *et al.* (*Animal Science and Technology*, 65(4):361-367 (1995)) in view of Rideout *et al.*

Teachings of the Cited References

Wang *et al.*

As discussed above, Wang *et al.* teach the generation of ES-derived mice by tetraploid blastocyst injection.

Wang *et al.* do not teach or suggest the use of non-inbred pluripotent cells, including non-inbred ES cells, in the generation of mutant and non-mutant mice. Wang *et al.* do not teach or suggest the generation of XO F1 (non-inbred) ES cells or their use in the generation of mutant and non-mutant mice.

Rideout *et al.*

As discussed above, Rideout *et al.* teach the generation of ES-derived mice by nuclear cloning using F1 (non-inbred) ES cells.

Rideout *et al.* do not teach or suggest the generation of mice using tetraploid blastocyst injection. Rideout *et al.* do not teach or suggest a method of producing XO F1 ES cells. As such, Rideout *et al.* do not cure the deficiencies of the Wang *et al.* and Uchida *et al.* references.

Uchida *et al.*

Uchida *et al.* is cited by the Examiner as teaching "the generation of XO ES cell line from an XY type ES cell line due to loss of the Y chromosome and its use in the generation of a mouse." Paper No. 8, at page 12, lines 20-21. Importantly, Uchida *et al.* suggest the use of their isolated ES cell line in producing female germ-line chimeras.

Uchida *et al.* do not teach or suggest the use of non-inbred pluripotent cells, including non-inbred ES cells, in the generation of mutant and non-mutant mice. Uchida *et al.* do not teach or suggest the generation of mice using tetraploid blastocyst injection and without the need to first create chimeric intermediates. Uchida *et al.* do not teach or suggest the generation of XO F1 ES cells or their use in the generation of mutant and non-mutant mice.

Combination of References

In support of the rejection, the Examiner alleges that:

> [I]t would have been obvious to one of ordinary skill in the art at the time the invention was made, to use the method of Uchida et al. (1995) to generate XO F1 ES cells using the male F1 ES cells as suggested by Rideout et al. (2000) to inject tetraploid blas[t]ocysts and generate mice, with a reasonable expectation of success. The motivation to do so was provided by Rideout et al. (2000) who teach that genetic background is probably an important factor in cloning efficiency and establish that F1 ES cells are efficient donor cells for generating cloned mice . . . and that manipulating the F1 ES cells in vitro before cloning may allow difficult questions such as the collective role of imprinted genes in mammalian development to be addressed . . . and by Wang et al. (1997) who teach that tetraploid blastocyst injection is an efficient way to minimize the and/or restrict the developmental potential of the host cells.

Paper No. 8, at page 13, lines 7-19.

As in the 103 rejection above, the Examiner has not identified a suggestion in the prior art of the desirability of the proposed combination of references, and as such, the present rejection is improper. The basis in the Rideout *et al.* reference relied upon by the Examiner for the desirability of the proposed combination of references would not have led one of ordinary skill in the art to combine the teachings of Rideout *et al.* with the teachings of Wang *et al.* and Uchida *et*

al. to obtain the claimed invention. The basis in the Wang *et al.* reference relied upon by the Examiner for the desirability of the proposed combination of references would not have led one of ordinary skill in the art to combine the teachings of Wang *et al.* and Uchida *et al.* with the teachings of Rideout *et al.* to obtain the claimed invention. The only document of record which suggests the desirability of the proposed combination is Applicants' specification. However, the use of the present specification as an instruction manual or template to piece together the teachings of the prior art is impermissible hindsight.

A *prima facie* case of obviousness is established only if the teachings of the cited art would have suggested the claimed invention to one of ordinary skill in the art with a reasonable expectation of successfully achieving the claimed results. In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Both the suggestion and the reasonable expectation of success must be found in the prior art, not Applicants' disclosure. Id.

Claims 42-44 and 46-48 relate to methods of producing a mutant mouse that is derived from a single non-inbred ES cell clone by breeding a mutant male mouse and a mutant XO female mouse produced from the same non-inbred male ES cell. Claim 44 relates to a method of producing XO F1 ES cells by introducing a negative selection marker into the Y chromosome of male F1 ES cells and selecting for male F1 ES cells that lack a Y chromosome. Claim 45 relates to an XO female mouse produced by introducing XO F1 ES cells into tetraploid blastocysts to produce an embryo that is transferred into a foster mother and permitted to develop to term.

None of the cited references (Wang *et al.*, Uchida *et al.* and Rideout *et al.*), alone or in combination, would have suggested the claimed invention to one of ordinary skill in the art at the time the invention was made with a reasonable expectation of success. In particular, one of ordinary skill in the art would not have been able to predict with a reasonable expectation of success, given the cited combination of references, whether a mutant mouse would be produced from a single non-inbred ES cell clone by breeding a mutant male mouse and a mutant XO female mouse produced from the same non-inbred male ES cell. One of ordinary skill in the art would not have been able to predict with a reasonable expectation of success, given the combination of cited references, whether XO F1 ES cells would be produced by introducing a negative selection marker into the Y chromosome of male F1 (non-inbred) ES cells and selecting for male F1 ES cells that lack a Y chromosome. One of ordinary skill in the art would not have

been able to predict with a reasonable expectation of success, given the combination of cited references, whether a XO female mouse would be produced by introducing XO F1 ES cells into tetraploid blastocysts to produce an embryo that is transferred into a foster mother and permitted to develop to term.

As discussed above, Wang *et al.* teach the generation of ES-derived mice by tetraploid blastocyst injection. Uchida *et al.* teach the generation of an XO ES cell line from an XY cell due to loss of the Y chromosome and its use in the generation of female chimeras. Neither Wang *et al.* nor Uchida *et al.* teach or suggest using non-inbred pluripotent cells, such as non-inbred ES cells (e.g., XO F1 ES cells), to generate mice. Neither Wang *et al.* nor Uchida *et al.* teach or suggest producing XO F1 ES cells. Rideout *et al.* teach the generation of ES-derived mice by nuclear cloning using F1 (non-inbred) ES cells, but do not teach or suggest the generation of mice using tetraploid blastocyst injection. As such, Rideout *et al.* do not cure the deficiencies of the Wang *et al.* and Uchida *et al.* references. Accordingly, the cited combination of references would not have suggested the claimed invention to one of ordinary skill in the art, at the time the invention was made, with a reasonable expectation of success. At best, the cited references merely indicate that specific isolated elements and/or features recited in the claims are known. This is insufficient to render the claimed invention *prima facie* obvious.

As discussed above, even assuming, *arguendo*, that a *prima facie* case of obviousness exists, which it does not, a patent applicant can rebut a *prima facie* case of obviousness by a showing of "unexpected results", e.g., by showing that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the art would have found surprising or unexpected. See, e.g., In re Soni, 34 U.S.P.Q.2d 1684, 1687 (Fed. Cir. 1995).

The production of XO mice involves long-term passage of the non-inbred ES cells in culture. The fact that live, adult mice, entirely derived from ES cells can be generated from F1 ES cells even after long-term passage of the cells in culture was unexpected and surprising, particularly since, as reported in the specification, it has previously been shown that continued passage of ES cells is detrimental to their developmental potency (see, e.g., page 15, lines 22-24).

Accordingly, even assuming, *arguendo*, that a *prima facie* case of obviousness exists, which it does not, it has been overcome by Applicants' unexpected results. Reconsideration and withdrawal of the rejection of Claims 42-48 under 35 U.S.C. § 103(a) are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

6. (Amended) The method of claim 5, wherein the non-human non-inbred ES cells are mouse non-inbred ES cells and the non-human mammalian embryo is a mouse embryo.

7. (Amended) The method of claim 6, wherein the mouse non-inbred ES cells are mutant mouse non-inbred ES cells and are injected into non-human tetraploid blastocysts by piezo microinjection.

40. (Amended) A method of identifying a candidate drug to be administered to treat a condition in a mammal in which [the] said condition occurs, comprising producing, using the method of claim 14, a mutant mouse that is a model of [the] said condition; administering to the mutant mouse a drug to be assessed for its effectiveness in treating or preventing [the] said condition; assessing the ability of the drug to treat or prevent [the] said condition, wherein if the drug reduces the extent to which [the] said condition is present or progresses, the drug is a candidate drug to be administered to treat [the] said condition.

41. (Amended) A method of producing a mutant non-human mammal, [mammal,] wherein pluripotent cells comprising at least one mutation in genomic DNA are introduced into tetraploid blastocysts of the same mammalian species under conditions that result in production of an embryo and the resulting embryo is transferred into a foster mother which is maintained under conditions that result in development of live offspring, wherein the pluripotent cells are non-inbred pluripotent cells.

44. (Amended) A method of producing mammalian XO F1 ES cells, comprising introducing into mammalian male F1 ES cells a negative selection marker, under conditions appropriate for insertion of the negative selection marker in the Y chromosome of mammalian male F1 ES cells, thereby producing a mixture of mammalian male F1 [Es] ES cells comprising male F1

ES cells in which the negative selection marker in inserted in the Y chromosome and other male F1 ES cells, some of which do not contain a Y chromosome; subjecting the resulting mixture to conditions that result in the death of male F1 ES cells in which the Y chromosome has the negative selection marker inserted therein and do not result in the death of male F1 ES cells that lack a Y chromosome and are XO F1 ES cells, thereby producing mammalian XO F1 ES cells.